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Changes in Salivary Amino Acid Composition During Aging

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Abstract. *Background: It has been suggested that the features of saliva reflect the physiological and psychological state of primates as well as subprimates, however, studies revealing the relationship between aging and the concentrations of salivary amino acids are limited. In order to better understand their physiological role, age-related changes of salivary amino acids were investigated. Materials and Methods: Forty-five participants including 5 children [6.60±1.67 (5-9) years old], 20 adults [46.55±14.68 (23-64) years old), and 20 senior citizens [71.60±4.27 (66-82) years old] took part in this study. Whole saliva (one sample per each person) was collected in the daytime (10:00-11:00 or 14:00-15:00). Salivary amino acids were recovered after deproteinization with 5% trichloroacetic acid and determined by an amino acid analyzer. Results: Glycine was the most abundant amino acid in the saliva. Glycine and lysine levels increased significantly ($p<0.05$) with aging, regardless of gender difference. When the glycine and lysine levels were plotted, much higher correlation ($p<0.001$) was observed. On the other hand, there was no significant correlation between the salivary concentration of glutamic acid or histidine and age. Conclusion: Salivary amino acid levels may be regarded as markers of aging.*

Saliva contains various physiologically active substances and cells that maintain homeostasis. Several amino acids in saliva may affect biological responses. Caries-free adults show elevated levels of lysine and arginine in the saliva, as compared with caries-susceptible adults (1). A significant relationship has been reported between the concentration of ammonium and caries prevalence (2). Glycine stimulated the production of prostaglandin E_2 and cyclooxygenase-2 protein in interleukin-1β-stimulated human gingival fibroblast (3). Supplementation of pigs with tryptophan in the diet reduced the basal plasma cortisol and noradrenaline concentrations (4). The major volatile substance of tobacco smoke, acetaldehyde, easily dissolves into saliva during smoking. This acetaldehyde can be totally removed by a cysteine-containing tablet which is sucked during smoking (5). Endogenous glutamate may alter hedonic response to suprathreshold umami substances (6). There are specific receptors for inhibitory amino acids [glycine and γ-aminobutyric acid (GABA)] and stimulatory amino acids (glutamic acid) in taste buds, and these amino acids exert their effects *via* their respective receptors (7-9). Changes in salivary composition correlate with disease susceptibility, disease state, or both. However, the use of saliva for diagnostic purposes is complicated by the gland-specific effects of circadian rhythm or diurnal variation, and therefore, studies of the circadian rhythm of saliva amino acid concentrations have been limited (10, 11). We recently investigated the diurnal changes of the salivary amino acids in three undergraduate students (12). The results showed that there was no apparent changes in most of salivary amino acid levels except for during eating and sport, and the ratio of glycine/glutamic acid declining under stressful condition during examinations (12). This study suggests that salivary amino acid levels may be useful to evaluate stressful conditions. In order to better understand their physiological role, we investigated here the possible changes in the salivary amino acid composition during aging.

Materials and Methods

Collection of saliva. Saliva was collected from a total of 45 people including 5 children [mean age 6.60±1.67 (5-9) years, 2 males and 3 females], 20 adults [mean age 46.55±14.68 (23-64) years, 16 males and 4 females), and 20 senior citizens [mean age 71.60±4.27 (66-82) years, 16 males and 4 females] who had medical examinations in Meikai University Hospital, according to the Guideline of the Intramural Ethics Committee (approved as No. A0842).

Determination of free amino acids. Whole saliva (one sample per each person) was collected into a beaker for 5 minutes in the daytime (10:00-11:00 or 14:00-15:00). Saliva (0.1 ml) was mixed with 0.1 ml

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of 10% trichloroacetic acid (Wako Pure Chem Co., Tokyo, Japan). After centrifugation for 5 minutes at 21,000× *g* at 4˚C, the deproteinized supernatant was collected and stored at –30˚C. The supernatants (20 μl) were subjected to a JLC-500/V amino acid analyzer (JEOL, Tokyo, Japan) and amino acids were detected by the ninhydrin reaction (13).

Statistical analysis. Statistical treatments were performed to delineate mean values, standard deviations and correlation coefficients, using the general linear model and ANOVA for multiple comparisons.

Results

Salivary amino acids of each participant are shown in Table I. Glycine was the most abundant amino acid in the saliva. The glycine level increased significantly (r^2=0.328, $p<0.05$) with aging, from children to senior citizens, to reach a maximum level of 571 μM (Figure 1). This trend did not change when the total population was divided into male and female groups.

Similarly, the salivary lysine level increased significantly (r^2=0.373, $p<0.05$) with aging, reaching a maximum level of 154 μM (Figure 2). This trend did not change when the total population was divided into male and female groups. When the glycine and lysine levels were plotted against each other, much higher correlation (r^2=0.847, $p<0.001$) was observed (Figure 3).

On the other hand, there was no significant correlation between the salivary concentration of glutamic acid (r^2=0.169) (Figure 4) nor that of histidine (r^2=0.201) and age (Figure 5).

Discussion

The present study demonstrated that salivary concentrations of glycine and lysine were significantly elevated during aging (Figures 1 and 2). This finding was not simply due to haemorrhage, since other amino acids, such as glutamic acid and histidine, did not show such correlation with age. Rajda *et al.* have reported that the saliva of migraineurs showed significantly higher concentration of glutamic acid, serine, glycine, arginine, and tyrosine as compared with control groups, suggesting that amino acids causing hyperexcitability in the central nervous system may be linked to the pathogenesis of migraine (14). Liappis *et al.* investigated the free amino acid concentration in the saliva of children with phenylketonuria, and reported that most of the amino acids including glycine are excreted much more poorly as compared with control groups (15). It has also been reported that lacto-ovo vegetarians had higher concentrations of salivary amino acids, including glycine, as compared with the control group (16). However, these previous studies have not investigated the relationship between these amino acid changes and aging.

We also found that inhibitory amino acid, GABA, was detectable only in persons of more than 65 years old (2 males and 2 females) (Table I). Among these people, three showed symptoms of hypertension, and one with moderate stages of periodontal diseases. There are also several reports that indicate the correlation between salivary amino acids and periodontal diseases (17-19), and between caries and salivary amino acid levels (1, 20). However, none of these

Table I. *Age-related salivary amino acid concentrations.*

	Salivary amino acid (μM)				
Age (years)	Gly	GABA	Glu	His	Lys
Male					
5	29.2		21.7	6.3	
5	7.5		5.4		
23	335.3		46.5	15.7	27.8
28	93.0		14.4	7.1	22.5
28	61.8		11.6	12.5	12.3
29	29.2		16.3	6.9	6.1
31	32.9		5.1	5.2	8.7
32	30.8		17.8	11.2	10.3
34	59.2				
38	21.9		5.6		
49	40.8		12.5	7.6	9.1
49	18.6		7.9		
54	10.0				
55	77.8			7.3	10.9
60	341.3			25.3	70.0
62	50.7			4.8	18.9
64	128.7		27.6	47.6	
64	77.7		16.4		33.8
66	36.4		22.0		15.9
66	51.9	7.2	44.9	14.9	8.8
67	116.2		14.1	14.7	22.0
67	101.4		11.6	8.8	15.2
69	94.7		14.4	18.2	18.6
70	28.8		14.0	4.9	9.0
71	64.5			9.2	14.9
72	117.7		4.6	5.2	28.2
72	160.7		12.1	11.1	20.3
72	43.6		5.5	11.9	5.1
74	277.1		13.6	31.7	43.4
74	21.6		4.5		
75	68.3	35.6	36.2	13.3	16.4
77	157.0		8.7	6.0	23.2
77	571.1		44.1	49.1	154.5
79	58.1		38.1	9.0	10.6
Female					
7	75.5		11.3	19.4	7.6
7	16.1			5.1	
9	27.8			14.6	5.5
46	126.3		5.9	8.6	11.8
60	217.2		8.6	13.8	35.5
61	278.7		70.5	17.9	59.9
64	209.9		9.6	17.9	38.6
69	13.7		5.1		
75	529.0	10.5		15.0	42.0
76	32.5		6.8		6.1
82	309.9	6.3	35.0	20.1	94.8

Blank data represent no detection of analyte.

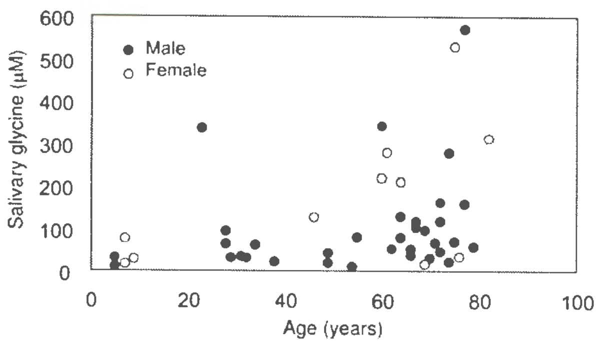

Figure 1. *Changes in salivary glycine concentration during aging. Amino acids from males (●) and females (○) were subjected to amino acid analysis. Good correlation was found between glycine level and age (r^2=0.328, p<0.05).*

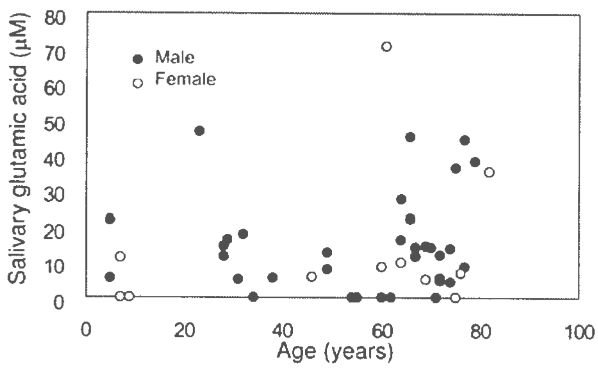

Figure 4. *Changes in salivary glutamic acid concentration during aging. Amino acids from males (●) and females (○) were subjected to amino acid analysis. There was no significant correlation between glutamic acid level and age (r^2=0.169).*

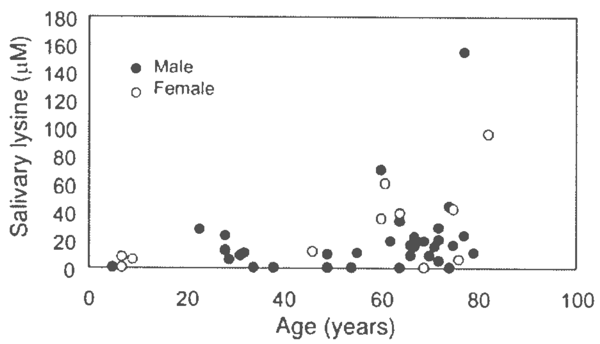

Figure 2. *Changes in salivary lysine concentration during aging. Amino acids from males (●) and females (○) were subjected to amino acid analysis. Good correlation was found between lycine level and age (r^2=0.373, p<0.05).*

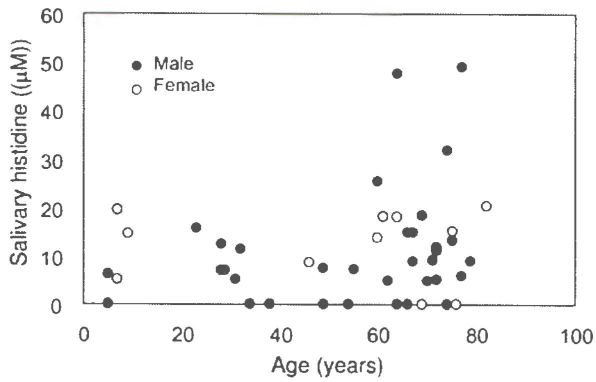

Figure 5. *Changes in salivary histidine concentration during aging. Amino acids from males (●) and females (○) were subjected to amino acid analysis. There was no significant correlation between histidine level and age (r^2=0.201).*

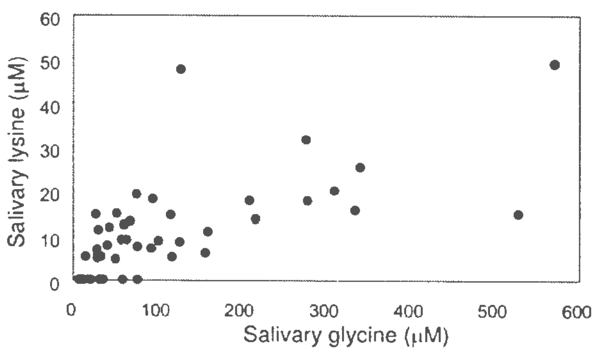

Figure 3. *Correlation between salivary glycine and lysine concentrations. Good correlation was found between levels of these two amino acids (r^2=0.847, p<0.001).*

reports has paid attention to the glycine level. We recently reported that mouse macrophage-like cells (RAW264.7, J774.1) produced higher amounts of glycine when activated by lipopolysaccharide (LPS), accompanied by elevated serine consumption (21). This suggests that the activated macrophages in the inflammatory tissues may be donors of glycine. Further studies are needed to identify the donor cells of amino acids in the oral cavity of periodontal disease patients and senior citizens. However, since the sample size in our study was small, further studies are recommended on a larger sample size with a longer period of surveillance.

In conclusion, the present study demonstrates that salivary glycine and lysine levels appear to increase during aging, regardless of gender, and there was significant correlation between the levels of these two amino acids.

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